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## ORIGINAL ARTICLE

# The association of genetic variants in the cholesteryl ester transfer protein gene with hemostatic factors and a first venous thrombosis

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## Abstract

**Background:** Cholesteryl ester transfer protein (CETP) plays an important role in lipoprotein metabolism. Previous studies have suggested that the *CETP TaqI* B1/B2 allele is associated with the risk of venous thrombosis (VT).

**Aim:** To investigate the associations between genetically determined CETP concentrations and 22 hemostatic factors in healthy individuals, and the risk of a first VT event, in a large VT case-control study.

**Methods:** Analyses were performed in the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) case-control study. CETP unweighted/weighted genetic risk scores (GRSs) were derived from three single-nucleotide polymorphisms that were identified from a recent genome-wide association study on serum CETP concentrations. The associations between CETP GRSs and 22 hemostatic factors (procoagulant/anticoagulant and fibrinolytic factors) were assessed by linear regression from an additive model in controls (n = 2813). The associations between CETP GRSs and the risk of a first VT were assessed by logistic regression analyses in 3950 VT cases and 4765 controls.

**Results:** In the controls (median age, 49 years; 53% women), both unweighted and weighted GRSs showed that factor VII activity was negatively associated with the genetically determined CETP concentration (weighted GRS  $\beta$  -3.08 IU/dL per  $\mu$ g/mL genetically determined CETP, 95% confidence interval -5.73 to -0.42). No association was observed with the risk of a first VT.

**Conclusions:** Genetically determined CETP concentrations only showed a weak negative association with factor VII activity. However, this did not lead to an association with the risk of a first VT.

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## KEYWORDS

anticoagulants, blood coagulation factors, cholesteryl ester transfer protein, Mendelian randomization analysis, polymorphism, single nucleotide, venous thrombosis

## 1 | INTRODUCTION

Cholesteryl ester transfer protein (CETP) plays an important role in lipoprotein metabolism, facilitating the net flux of cholesteryl esters from high-density lipoproteins (HDLs) towards low-density lipoproteins (LDLs) and very-low-density lipoproteins.<sup>1</sup> As elevated CETP concentrations contribute to an adverse lipoprotein profile, and high HDL cholesterol (HDL-C) levels have been associated with a reduced risk of cardiovascular disease (CVD),<sup>2</sup> CETP inhibitors have been developed with the aim of reducing the risk of CVD.<sup>3,4</sup> However, despite the fact that all CETP inhibitors increased HDL-C levels, only one of the CETP inhibitors that have been tested extensively in humans showed a significant, although small, benefit regarding the risk of CVD.<sup>5</sup> Thus, the biological role and function of CETP remain to be further investigated.

The first association between CETP and the risk of venous thrombosis (VT) was described by Deguchi et al, who showed that the frequency of the CETP *TaqI* B2 allele, which was associated with decreased plasma levels of CETP antigen and activity, was lower in VT cases than in controls.<sup>6</sup> However, the study was performed only in men, and had a small number of individuals with VT. In another recent study on the association between plasma CETP activity and coagulability, a negative correlation was observed between plasma CETP activity and activated partial thromboplastin time, suggesting an association between CETP activity and plasma coagulability.<sup>7</sup> The mechanism of CETP procoagulant activity was postulated to be related to the direct binding of CETP to activated factor X (FXa) with enhanced prothrombinase activity.<sup>7,8</sup>

A genome-wide association study (GWAS) was recently performed to identify genetic variants that are associated with serum CETP concentrations.<sup>9</sup> From this GWAS, three independent single-nucleotide polymorphisms (SNPs), i.e., hg19 chr16:g.56989590C>T, chr16:g.57000885A>G, and chr16:g.57010948G>T, were identified and were strongly associated with serum CETP concentrations, with effect sizes ranging from 0.32 to 0.12  $\mu\text{m}/\text{mL}$  changes in CETP concentrations for the addition of one risk allele.<sup>9</sup> The newly identified SNPs shared moderate linkage disequilibrium (LD) with the previously used CETP variants in the literature (e.g., *TaqI* B1/B2, Arg451Gln, and Ala373Pro). Altogether, the three SNPs explained some 16% of variation in the CETP concentration. This opens the possibility of performing Mendelian randomization (MR) studies to investigate the causal relationships between CETP concentrations, the levels of hemostatic factors in plasma, and disease risk.<sup>10</sup>

In the current study, we aimed to use the three newly identified CETP SNPs as genetic instrumental variables to explore

### Essentials

- *TaqI* B1/B2 allele in CETP gene has been associated with the risk of venous thrombosis (VT) in males.
- Whether CETP is a risk factor for VT is still unclear.
- We assessed the associations between CETP genotype and 22 hemostatic factors and the risk of a first VT.
- Genetically determined CETP concentration only showed a weak negative association to factor VII activity.
- No association was found with a higher risk of a first VT.

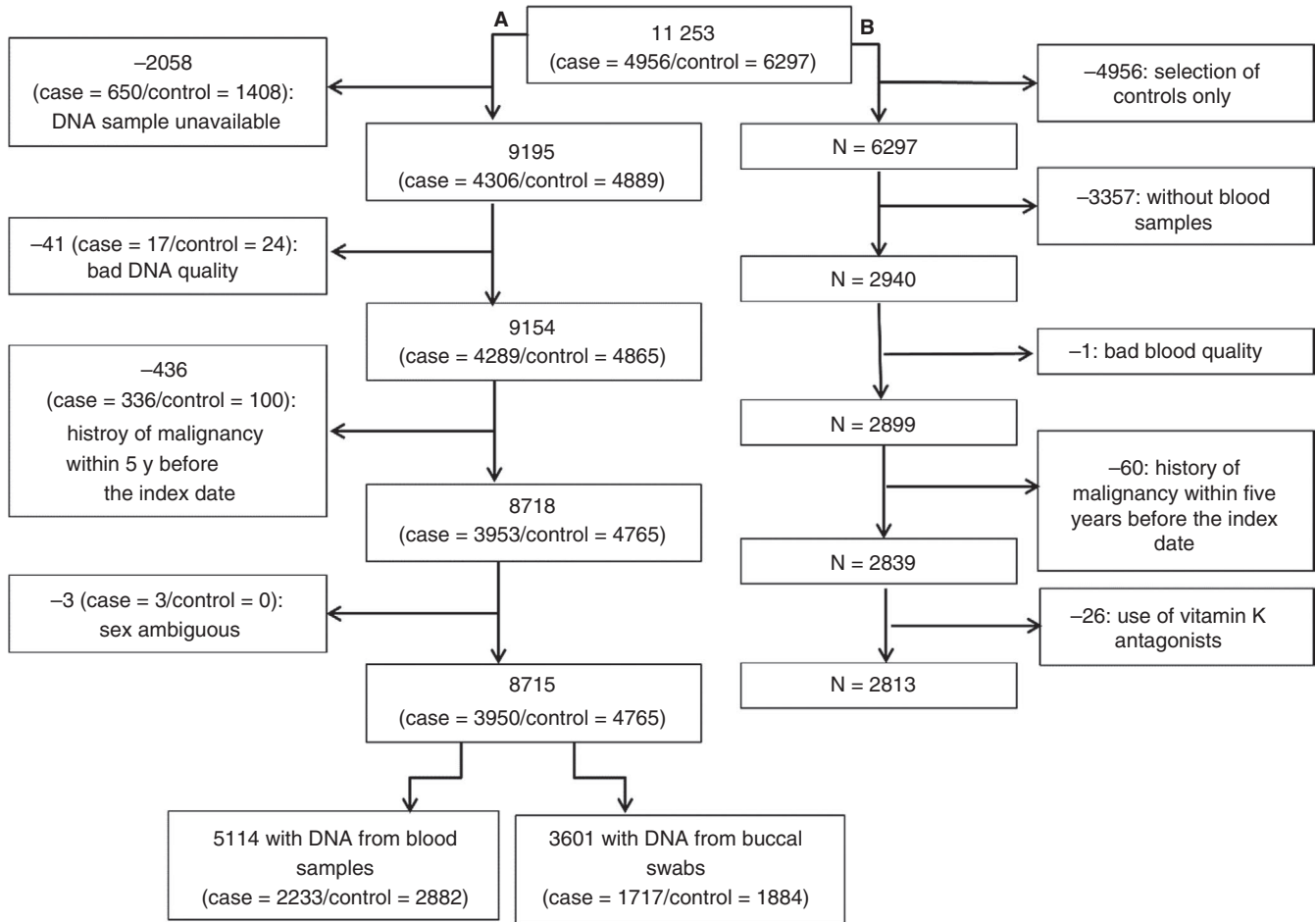
the associations between genetically determined CETP concentrations and (a) hemostatic factors, including procoagulant, anticoagulant and fibrinolytic factors, and (b) the risk of a first VT in a large, population-based case-control study, the Multiple Environmental and Genetic Assessment (MEGA) of Risk Factors for VT study.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

The MEGA study is a population-based case-control study with the aim of studying the etiology of VT. The study design was approved by the Ethics Committee of the Leiden University Medical Center, the Netherlands, and written informed consent was obtained from all participants. From March 1999 to September 2004, 4956 consecutive patients aged between 18 and 70 years (>90% of European ancestry) with an objectively confirmed first VT or pulmonary embolism (PE) event were recruited from six anticoagulation clinics in the Netherlands.<sup>11</sup> The control subjects were recruited from two sources: partners of VT patients when they were between 18 and 70 years of age and without a history of VT ( $n = 3297$ ); and from the general population, by random-digit dialing. They were frequency-matched for age and sex with the VT cases ( $n = 3000$ ). For logistic reasons, a blood sample was provided only by patients and controls recruited before June 2002. For the participants who were not available for a blood draw, buccal swabs were collected for DNA analysis. In total, approximately 60% of DNA material was extracted from blood samples, and 40% from buccal swabs (Figure 1).

For the analyses on the associations between genetically determined CETP concentrations and hemostatic factors, the following participants were selected: controls with a blood sample available,



**FIGURE 1** Flowchart of sample selection from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) study. A, The selection for the analysis of cholesteryl ester transfer protein (CETP) genetic risk scores (GRSs) and the risk of venous thrombosis. B, The selection for the analysis of CETP GRSs and hemostatic factors

without a history of malignancy within 5 years before the index date, and not using vitamin K antagonists (Figure 1B). For the analyses on the association between genetically determined CETP concentrations and the risk of a first VT, participants were selected with DNA samples available and of good quality, without a history of malignancy within 5 years before the index date, and without Klinefelter syndrome (Figure 1A).

## 2.2 | Laboratory measurements

Detailed information on laboratory measurements of hemostatic factors in the MEGA study is available elsewhere.<sup>12</sup> In brief, FII activity, FVII activity, FVIII activity, FX activity and FXI activity were measured with a mechanical clot detection method on an STA-R coagulation analyzer (Diagnostica Stago), to quantify the potentials of generating active coagulation factors capacity on the assay. FV levels were determined by use of an in-house-developed sandwich ELISA with two mAbs recognizing the light chain (V-6) or the heavy chain (V-39) of FV, which was specific for single-chain FV, including FV-short.<sup>13</sup> FIX antigen levels were

determined by use of an in-house ELISA with rabbit anti-FIX antibodies and anti-FIX IgG conjugated to horseradish peroxidase (Dako A/S).<sup>14</sup> Antithrombin activity and protein C levels were measured with a chromogenic assay on an STA-R coagulation analyzer, according to the manufacturer's instructions (Diagnostica Stago).<sup>15</sup> Total protein S levels were measured with an ELISA (Diagnostica Stago). Plasminogen activator inhibitor-1 (PAI-1) antigen levels were measured with a Technozym PAI-1 ELISA reagent kit (Kordia Life Sciences; Biopool), and D-dimer levels were measured with the HemosIL D-dimer assay on an ACL TOP 700 analyzer.<sup>16</sup> Clot lysis time (CLT) was derived from a clot lysis turbidity profile, which has been described previously with details.<sup>12</sup> Several fibrinolytic factors were measured in only a subsample of the MEGA study, including PAI-1, tissue-type plasminogen activator, plasminogen and antiplasmin ( $\alpha_2$ -antiplasmin) concentrations, and thrombin-activatable fibrinolysis inhibitor activity. All of the laboratory measurements were performed without knowledge of VT case-control status. The measurements of PAI-1, D-dimer and CLT were logarithm-transformed to obtain normal distributions.

## 2.3 | SNP genotyping and genetic risk score

Three SNPs (rs247616:C>T, rs12720922:A>G, and rs196890:G>T) identified from the previous CEPT GWAS<sup>9</sup> were genotyped in the MEGA study. DNA samples were obtained from blood samples or buccal swabs, with a concentration of 3 ng/μL. Genotyping of individual DNA samples was performed with kPCR assays using 0.3 ng of DNA or multiplexed oligonucleotide ligation assays. The genotyping accuracy of both systems has been assessed in previous studies, and the concordance of the genotype calls from these methods was >99%.<sup>17-19</sup>

An unweighted and a weighted genetic risk score (GRS) were calculated. The unweighted GRS was defined as the counts of the total number of CETP concentration-increasing (risk) alleles. To take the SNP effect size into account, the weighted GRS was calculated as the sum of numbers of CETP concentration-increasing alleles multiplied by the SNP effect sizes reported from the original GWAS.

Weighted GRSs were considered to be the least biased estimates for the genetically determined CETP concentration.

## 2.4 | Statistical analyses

Linear regression models were used to estimate the effect sizes ( $\beta$ ) with 95% confidence intervals (CIs) for the associations of both individual SNPs and the derived CETP GRSs (both weighted and unweighted) with 22 hemostatic factors.  $\beta$  can be interpreted as the difference in hemostatic factor measures (units used in the measures) per unit (μg/mL) of genetically determined CETP concentration. For the hemostatic factors that were logarithmically transformed (namely, D-dimer, CLT, and PAI-1),  $\beta$  represents the percentage change in the factor measures per unit (μg/mL) of genetically determined CETP concentration. For this analysis, extreme measures of each of the 22 hemostatic factors were excluded, i.e., when the individual value was beyond five standard deviations from the mean. To correct for

**TABLE 1** Baseline characteristics

Characteristics	Association with hemostatic factors in controls with blood samples (n = 2813)	A first VT event	
		Cases (n = 3950)	Controls (n = 4765)
Age (y), median (IQR)	49 (39-58)	49 (38-58)	49 (39-58)
Women, n (%)	1490 (53)	2161 (54.7)	2536 (53.2)
BMI (kg/m <sup>2</sup> ), median (IQR)	25.1 (22.7-27.7)	26.3 (23.7-29.3)	25.1 (22.8-27.8)
DVT, n (%)	NA	2316 (58.6)	NA
PE, n (%)	NA	1263 (32.0)	NA
DVT and PE, n (%)	NA	371 (9.4)	NA
A first unprovoked VT, n (%)	NA	1149 (29.1) <sup>a</sup>	NA
A first provoked VT, n (%)	NA	2715 (68.7) <sup>a</sup>	NA
Total cholesterol (mmol/L), median (IQR)	5.5 (4.8-6.3)	5.5 (4.8-6.3)	5.5 (4.8-6.3)
Triglycerides (mmol/L), median (IQR)	1.3 (1.0-1.9)	1.4 (1.0-1.9)	1.3 (1.0-1.9)
HDL-C (mmol/L) median (IQR)	1.3 (1.1-1.6)	1.3 (1.0-1.5)	1.3 (1.1-1.6)
LDL-C (mmol/L), median (IQR)	3.5 (2.9-4.2)	3.5 (2.9-4.2)	3.5 (2.9-4.2)
rs247616_C			
Call rate (%)	98.4	79.5	83.4
Minor allele frequency (%)	32.3	27.8	27.1
HWE P value	0.78	NA	0.67
rs12720922_A			
Call rate (%)	99.3	92.6	95.3
Minor allele frequency (%)	16.7	15.8	15.3
HWE P value	0.49	NA	0.074
rs1968905_G			
Call rate (%)	99.5	92.5	95.6
Minor allele frequency (%)	19.9	17.5	17.5
HWE P value	0.14	NA	0.33

Abbreviations: BMI, body mass index; DVT, deep vein thrombosis; HDL-C, high-density lipoprotein cholesterol; HWE, Hardy-Weinberg equilibrium; LDL-C, low-density lipoprotein cholesterol; NA, not applicable; PE, pulmonary embolism.

<sup>a</sup>Eighty-six VT patients with unknown provoking factors.

multiple testing, the significance level was set to  $P < 0.0083$ ; the standard significance level ( $P = 0.05$ ) was divided by the number ( $N = 6$ ) of principal components that explain over 95% of the variation of 22 hemostatic factors. To identify the associations between genetically determined CETP concentrations and the risk of a first VT, logistic regression models were applied to estimate the odds ratios (ORs) with 95% CIs for both the single SNPs and the weighted/unweighted CETP GRSs.

In addition to the analysis on the total population, a stratified analysis was performed for men and women. The risk of VT

associated with genetically determined CETP concentrations was estimated for provoked and unprovoked VT events separately. Unprovoked thrombosis was defined as the absence of any of the following provoking factors: surgery, trauma, hospitalization, immobilization, plaster cast use, hormone use (oral contraceptives and hormone therapy), pregnancy within 3 months before the first event, being within 4 weeks postpartum, and long-haul flight (>4 hours) in the 2 months before the first thrombosis.<sup>20</sup> Similarly, separate analyses on the risks of deep vein thrombosis (DVT) and PE were performed for both the single SNPs and CETP GRSs.

**TABLE 2** The association of cholesteryl ester transfer protein (CETP) concentration genetic risk scores (GRSs) with coagulation factors as determined with additive models

	Unweighted GRS			Weighted GRS		
	N	$\beta$	95% CI	N	$\beta$	95% CI
Anticoagulant factors						
Protein C activity (IU/dL)	2753	-0.30	-0.99 to 0.39	2753	-1.31	-3.63 to 1.00
Total protein S antigen (IU/dL)	2753	0.17	-0.46 to 0.80	2753	0.69	-1.42 to 2.80
Free protein S (%)	2725	0.63	-0.054 to 1.32	2725	1.66	-0.66 to 3.97
TFPI activity (U/mL)	2752	-0.0060	-0.020 to 0.0090	2752	-0.019	-0.069 to 0.031
Antithrombin activity (IU/dL)	2750	-0.12	-0.47 to 0.24	2750	-0.55	-1.75 to 0.65
Procoagulant factors						
Fibrinogen activity (g/L)	2748	0.0050	-0.015 to 0.026	2748	0.020	-0.049 to 0.089
Factor II activity (IU/dL)	2751	-0.019	-0.48 to 0.44	2751	-0.17	-1.71 to 1.37
Factor V antigen (U/mL)	2749	0.0020	-0.0040 to 0.0070	2749	0.0020	-0.016 to 0.020
Factor VII activity (IU/dL)	2753	-0.92	-1.71 to -0.13	2753	-3.08	-5.73 to -0.42
Factor VIII activity (IU/dL)	2750	1.00	-0.19 to 2.19	2750	4.26	0.26-8.26
Von Willebrand factor antigen (IU/dL)	2747	0.72	-0.62 to 2.07	2747	2.99	-1.53 to 7.51
Factor IX antigen (IU/dL)	2752	-0.053	-0.65 to 0.55	2752	-0.29	-2.31 to 1.74
Factor X activity (IU/dL)	2752	0.12	-0.50 to 0.74	2752	0.13	-1.96 to 2.23
Factor XI activity (IU/dL)	2752	0.061	-0.56 to 0.68	2752	0.24	-1.85 to 2.33
Global assay measurements						
Clot lysis time (min) <sup>a</sup>	2747	0.40	-0.30 to 1.21	2747	1.21	-1.29 to 3.87
ELPLT (nM.min)	2741	-0.30	-4.50 to 3.90	2741	-1.43	-15.57 to 12.70
Fibrinolytic factors						
PAI-1 (ng/mL) <sup>a</sup>	704	0.90	-3.82 to 5.76	704	5.97	-9.79 to 24.61
t-PA (ng/mL)	704	0.057	-0.086 to 0.20	704	0.38	-0.10 to 0.86
D-dimer (ng/mL) <sup>a</sup>	2749	0.40	-1.69 to 2.43	2749	1.41	-5.26 to 8.55
Plasminogen concentration (%)	702	-0.18	-1.22 to 0.86	702	-0.90	-4.38 to 2.58
$\alpha_2$ -Antiplasmin concentration (%)	702	0.056	-0.65 to 0.76	702	-0.11	-2.46 to 2.24
TAFI activity (%)	701	0.48	-0.65 to 1.60	701	1.12	-2.65 to 4.89

Abbreviations: CI, confidence interval; ETPLP, endogenous thrombin potential (area under the curve) obtained at low tissue factor concentrations; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; t-PA, tissue-type plasminogen activator.

<sup>a</sup>Natural logarithm-transformed, with  $\beta$  estimated as the percentage change in the factor measures per unit ( $\mu\text{g/mL}$ ) in the genetically determined CETP concentration.

A power calculation was performed based on the current study population (with 45% VT cases) with an online power calculator for MR,<sup>21</sup> and 5839 samples ( $N = 8715$  in the current study) were found to be needed to achieve 80% power by the settings of type I error rate ( $\alpha = 0.05$ , proportion of CETP concentration variance explained by the three SNPs ( $R^2_{xz}$ ) of 16%, and an OR of 1.2. All statistical analyses were performed with SPSS for Windows, release 23 (SPSS).

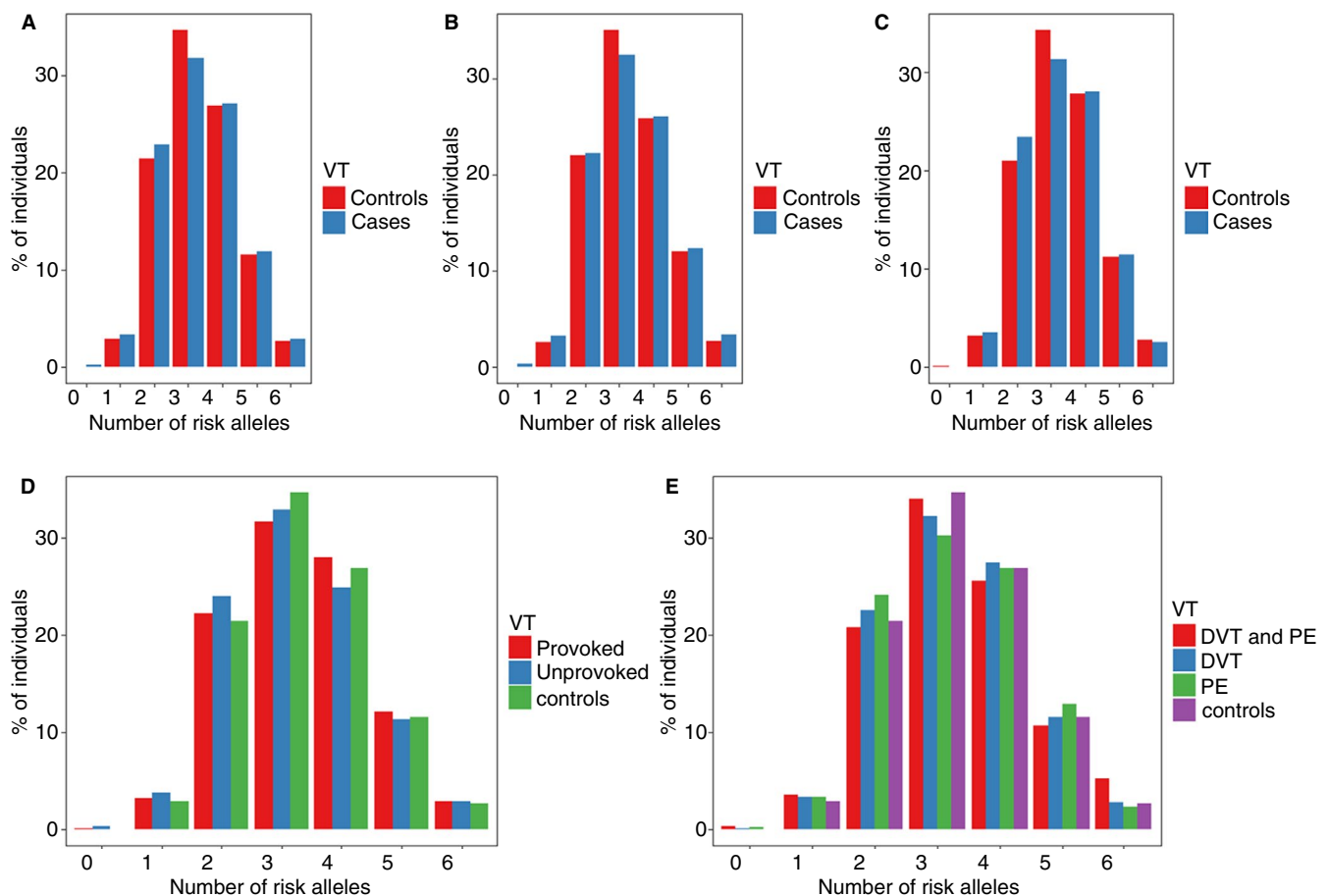
### 3 | RESULTS

Table 1 summarizes the baseline characteristics of all participants in both analyses. Among 2813 controls used for the hemostatic factor analyses, the minor allele frequencies of the three selected SNPs were similar to those in the previously published GWAS.<sup>9</sup> The median body mass index was higher in VT patients ( $26.3 \text{ kg/m}^2$ ) than in controls ( $25.1 \text{ kg/m}^2$ ) for the analysis of association with the risk of a first VT. Of all VT events, over 68% were provoked. VT patients and controls showed similar clinical lipid profiles, including total cholesterol, LDL cholesterol, HDL-C, and triglycerides. All three SNPs passed the Hardy-Weinberg equilibrium tests in controls ( $P > 0.05$ ). The call rates for all the three SNPs did not differ between VT cases

and controls, and the SNP calling missingness was mainly due to the weaker integrity of DNA obtained from buccal swabs than of blood-derived DNA (over 90% of missing SNP genotypes were from DNA samples obtained with buccal swabs).

We studied the association between CETP SNPs (both individually and using a GRS) and hemostatic factors and the risk of VT by using an MR approach, with the aim of investigating the causal relationships between CETP concentrations, the levels of hemostatic factors in plasma, and disease risk. The associations between individual genetic variants and the levels of hemostatic factors are shown in Table S1. After Bonferroni correction ( $P < 0.0083$ ), an association was observed between rs247616:C>T and FVII activity (that is, FVII activity decreased with a 1.93 IU/dL per  $\mu\text{g/mL}$  increase in the genetically determined CETP concentration:  $\beta = -1.93$ , 95% CI  $-3.24$  to  $-0.61$ ) (Table S1). Similarly, another two positive associations were found with the uncorrected significance level ( $P < 0.05$ ), between rs247616:C>T and FVIII activity ( $\beta = 2.34$  IU/dL per  $\mu\text{g/mL}$  genetically determined CETP concentration, 95% CI 0.37–4.32), and between rs12720922:A>G and CLT ( $\beta = 1.71\%$  per  $\mu\text{g/mL}$  genetically determined CETP concentration, 95% CI 0.20%–3.36%) (Table S1).

With the GRSs based on the three CETP SNPs, only weak associations were present (Table 2): FVII activity showed associations



**FIGURE 2** The three-single-nucleotide polymorphism risk allele distributions between venous thrombosis (VT) cases and controls. A, The entire cohort. B, Men only. C, Women only. D, VT cases separated into provoked and unprovoked VT. E, VT cases separated into pulmonary embolism (PE) and deep vein thrombosis (DVT)



**TABLE 3** The association of cholesteryl ester transfer protein concentration genetic risk scores (GRS) with the first venous thrombosis (VT) event as determined with additive models

	Unweighted GRS				Weighted GRS			
	N <sub>case</sub>	N <sub>control</sub>	OR	95% CI	N <sub>case</sub>	N <sub>control</sub>	OR	95% CI
All participants	3101	3906	0.99	0.95-1.03	3101	3906	0.98	0.85-1.13
Participants stratified by sex								
Men only	1406	1853	1.02	0.96-1.08	1406	1853	1.09	0.89-1.35
Women only	1695	2053	0.98	0.92-1.03	1695	2053	0.91	0.74-1.11
Provoked/unprovoked VT								
Provoked VT only	2130	3906	1.01	0.96-1.06	2130	3906	1.01	0.86-1.19
Unprovoked VT only	902	3906	0.95	0.89-1.02	902	3906	0.87	0.69-1.10
PE/DVT								
PE and DVT	309	3906	1.03	0.93-1.14	309	3906	1.04	0.73-1.49
DVT only	1804	3906	0.99	0.94-1.04	1804	3906	0.97	0.82-1.15
PE only	988	3906	0.98	0.92-1.05	988	3906	0.96	0.78-1.19

Abbreviations: CI, confidence interval; DVT, deep vein thrombosis; OR, odds ratio; PE, pulmonary embolism.

with both the weighted CETP GRS ( $\beta = -3.08$  IU/dL per  $\mu\text{g/mL}$  genetically determined CETP concentration, 95% CI  $-5.73$  to  $-0.42$ ) and the unweighted CETP GRS ( $\beta = -0.92$  IU/dL per  $\mu\text{g/mL}$  genetically determined CETP concentration, 95% CI  $-1.71$  to  $-0.13$ ). FVIII activity also showed an association with the weighted CETP GRS ( $\beta = 4.26$  IU/dL per  $\mu\text{g/mL}$  genetically determined CETP concentration, 95% CI  $0.26$ - $8.26$ ).

For each participant, the number of CETP concentration-increasing (risk) alleles was counted. The number of risk alleles ranged from zero to six (Figure 2), and similar proportions of VT cases and controls fell into each allele count category. In logistic regression models, neither single SNPs (Table S2) nor weighted and unweighted CETP GRSs (Table 3) were associated with an increased risk of a first VT, with an OR of 0.98 (95% CI 0.85-1.13) for the weighted CETP GRS in the entire study population (Table 3). No associations were observed in separate analyses on provoked and unprovoked VT; that is, ORs were 1.01 (95% CI 0.86-1.19) for provoked VT and 0.87 (95% CI 0.69-1.10) for unprovoked VT by the weighted CETP GRS, as well as for the separate analyses on PE and/or DVT. Stratified analysis on sex yielded the same null results.

## 4 | DISCUSSION

In the current large-scale population-based VT case-control study, of 22 hemostatic factors studied, the genetically determined CETP concentration was found to be weakly associated with FVII activity levels, and this was predominantly attributable to a single SNP (rs247616:C>T). However, no association was observed between genetically determined CETP concentrations and the risk of a first VT, which is in line with the absence of an association between FVII levels and the risk of VT in the literature.<sup>22</sup>

Deguchi et al first described the association of the CETP TaqI B1/B2 polymorphism (rs708272) with VT in a small case-control study

composed of 98 male participants,<sup>6</sup> but this was not observed in the current study. In this study, the frequency of the TaqI B2 allele was lower in VT cases than in controls (0.33 vs 0.47). Pecheniuk et al further investigated the allele frequencies of two other non-synonymous CETP SNPs (Ala373Pro and Arg451Gln) in the same case-control study population. For both variants, CETP-increasing alleles were more often found in male VT cases than in controls.<sup>23</sup> On the basis of this evidence, the authors suggested that CETP genotypes were associated with VT in men. However, in contrast to the previous findings, in the current VT case-control study with a much larger sample size, we found no association with the risk of VT in either men or women. This null association was found despite the observation that the GRS explained over 16% of the variation in CETP concentration.<sup>9</sup> The three SNPs used in the current analyses are in moderate LD with the previously used SNPs (pairwise LDs between 0.51 and 0.55), which is unlikely to explain the difference in results from those of the previous studies. However, it is noteworthy that, in the previous case-control study with 98 male participants, the controls were recruited from a blood donation program, which is likely to recruit controls screened for good health, and in particular with beneficial lipid profiles.<sup>6</sup> As a result, selection bias might have been introduced into their study, which is in line with a higher allele frequency of TaqI B2 in the controls than in the Caucasian reference population from the 1000 Genome project (0.47 vs 0.42).<sup>24</sup>

A previous study showed inverse correlations of endogenous plasma CETP antigen levels with prothrombin time induced by tissue factor (reflecting the extrinsic coagulation pathways) and with activated FIX-induced clotting time (reflecting the intrinsic pathways), which implied a potential association between CETP and coagulability through a common pathway, namely prothrombin activation.<sup>25</sup> Another recent study further demonstrated that enhanced CETP prothrombin activation occurred through direct binding of CETP to FXa.<sup>7</sup> In addition, thrombin generation measured by the use of prothrombin activation assays was reported to be increased 5-fold in the presence



of the Gln451 (rs1800777 A/G, with CETP concentration-increasing allele A) CETP mutation than in the presence of wild-type CETP.<sup>7</sup> On the basis of this evidence, our null associations between the genetically determined CETP concentration and both prothrombin (FII) and FX activity were unexpected. Nevertheless, our results are in line with our previous findings showing no association between lipid levels and the risk of a first VT.<sup>26</sup> This is relevant, because CETP concentrations are highly inversely associated with HDL-C levels.<sup>27,28</sup> Previous findings have indicated associations between FVII and lipid profiles<sup>29</sup>; however, the FVII level is not a risk factor for VT in the literature.<sup>22</sup> Taken together, these findings may explain the association between genetically determined CETP GRSs and FVII levels as we observed in the current study. However, FVII level is not a risk factor for VT, which is in line with the absence of association with the risk of VT in the current study. In addition, thrombin generation was also measured in the current study according to the endogenous thrombin potential (area under the curve) obtained at low tissue factor concentrations (ETPLT), and a null result was obtained between ETPLT and CETP GRSs, questioning the previously observed associations between CETP genetic mutations and thrombin generation.

There are several strengths of the current study. First, to our knowledge, this is the largest study with extensive hemostatic factors measured in the study population to investigate the associations between CETP concentrations and both hemostatic factors and the risk of a first VT. Second, we used strong genetic instruments (explaining ~16% of the phenotypic variation) to reflect CETP concentrations, which increased the statistical power of the analyses. Third, by using the GRSs, we performed two-sample MR analyses, which are less vulnerable to reverse causation and residual confounding issues in observational studies. For etiologic studies, the associations observed in this study are more likely to be causal.

Several limitations of the current study should also be acknowledged. First, although it is well powered for the main analysis, the sample size might still be insufficient for the sex-stratified analysis and for the separate analyses on provoked/unprovoked VT and DVT/PE. Second, CETP GRSs were derived on the basis of Caucasian populations, and the findings of the current study might not apply to other ethnicities. Third, DNA extracted from buccal swabs had higher genotyping failure rates than DNA extracted from blood samples, because of decreased DNA integrity. However, the call rates for each SNP were similar between cases and controls, and the missingness is unlikely to be related to disease status.

In conclusion, genetically determined CETP concentrations showed weak associations with FVII activity. However, no association was found between genetically determined CETP concentrations and the risk of a first VT.

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## CONFLICT OF INTERESTS

D. O. Mook-Kanamori is a part-time clinical research consultant for Metabolon, Inc. All other authors have nothing to disclose.

## AUTHOR CONTRIBUTIONS

R. Li-Gao analyzed and drafted the manuscript. R. Li-Gao and A. van Hylckama Vlieg interpreted the data. F. R. Rosendaal and A. van Hylckama Vlieg designed the study. All authors reviewed the manuscript.

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## SUPPORTING INFORMATION

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